PHYSICOCHEMICAL AND THERMODYNAMIC CHARACTERISTICS OF BIOLOGICALLY ACTIVE SUBSTANCES EXTRACTED FROM PLANT MATERIAL BY SUPERCRITICAL FLUID EXTRACTION

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ABSTRACT

Low temperature adiabatic calorimetry methods in the region of 80-350 K, combustion calorimetry and differential thermal analysis in the region 80-650 K studied thermodynamic characteristics (heat capacity, and the standard enthalpy of formation and of combustion) and physicochemical properties (melting, crystallization, evaporation, vitrification) biologically active substances extracted from plant material by supercritical fluid extraction.

INTRODUCTION

For preparation of the biologically active substances (BAS) from the vegetable products have recently been used successfully extracting the gases that are in the supercritical state [1-4]. This method is environmentally friendly and refers to the "green chemistry". [5] For standardization BAS obtained by supercritical fluid extraction (SCF) carbon dioxide, require reliable data on their physical and chemical properties and thermodynamic characteristics [6]. Therefore, we of low temperature adiabatic calorimetry methods in the region of 80-350 K, combustion calorimetry and differential thermal analysis (DTA) in the region 80-650 K studied thermodynamic characteristics (heat capacity, and the standard enthalpy of formation and of combustion) and physicochemical properties (melting, crystallization, evaporation, vitrification) CO_2-extracts from shoots of pine (*Pinus sylvestris*), birch bark (*Betula pendula*) and betulin (BT) extracted from it, as well as plant oils from the annual geranium (*Pelargonium graveolens*) (No1), Abkhazian mandarin (*Citrus Unshiu*) (No2), basil (*Ocimum basilicum*) (No3), pomegranate seed (*Punica granatum*) (No5), St. John's wort (*Hypericum perforatum*) (No6). For comparison, oil was studied, as distinguished from the annual geranium (*Pelargonium graveolens*) using microwave extraction (No4). In addition, chromatographic and spectroscopic methods was investigated qualitative and quantitative composition of CO_2-extracts. Also studied the radioprotective, antiatherogenic and antioxidant properties of CO_2-extracts from pine shoots.

MATERIALS AND METHODS

The physicochemical properties of the samples BAS (from shoots of pine and birch bark) obtained by SKF patented technology [7] on the installation developed Scientific and Industrial "Grande" Ltd. (Nizhny Novgorod). The plant is designed to produce SKF extracts from raw vegetable and animal origin at pressures from 7.3 to 37 MPa and temperatures from 304 to 333 K. Performance in terms of dry feed 20 kg/h

Organoleptic CO_2-extracts of pine shoots [8] are thick pasty mass at room temperature with a typical pine smell and color from yellow to dark green. Their density is 1.00-1.03 g/cm^3. Kinematic viscosity is determined by a capillary viscometer at 323 K, equal to 900-1400 mm^2/s.

CO_2 extract of birch bark is white or slightly yellowish powder. Betulin is isolated from the extract was purified by recrystallisation three times from solution in chloroform. Previously we have determined the solubility at 296.35 K BT in chloroform (1.678 g $C_{30}H_{50}O_2$ per 100 g of solvent). Elemental analysis of the purified BT revealed that the content by wt.%: C − 81.67, H − 11.45, O − 6.88 (theory − 81.39, 11.38, 7.23, respectively). The purity of purified BT also determined by chromato-mass-spectrometry analysis (Fig.1). The experiment was conducted on the chromato-mass-spectrometer Trace GC Ultra/DSQII. Used capillary column TR 5 MS, 60 m long and 0.25 mm in diameter. A sample volume of 1 μl were injected into the chromatograph injector is heated to 583 K. The flow rate of carrier gas (helium) was 1 ml/min. The column temperature was varied from 473 to 583 K. The masschromatograms were recorded in the mass number range 35-600. Record the chromatograms started after 4 min (for clipping solvent peak). Identification of components of the mixtures was carried out using the electronic library massspectra, "NIST 2005". As can be seen from Fig.1, a mass-chromatogram is present only peak corresponding BT. The analysis showed that the content of impurities in the purified BT does not exceed 0.5 wt.%.

Conditions for obtaining a CO_2-extract plant oils (No1 – No3, No5 and No6): $p = 5.5\ 10^4$ kPa, $T = 313$ K, flow rate of 50 or 75 g/min. Samples prepared Kurnakov Institute of General and Inorganic Chemistry RAS

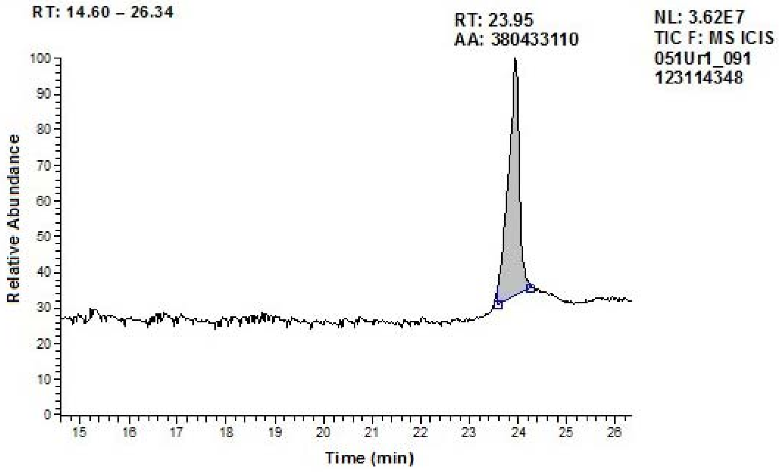

Fig.1. Mass-chromatogram of purified betulin

To measure the heat capacity (C_p°) of purified betulin ($m = 0.3226$ g) in the region of 80-350 K using a vacuum adiabatic calorimeter BKT-3.07 with discrete heating and maintaining automated adiabatic measurement conditions [9, 10]. The calorimeter is designed and manufactured in Joint-Stock Corp. "Termis" (Village Mendeleevo, Moscow reg.). The installation consists of a submersible type with minikriostata calorimetric device, analogue control unit and computer-measuring system "AK-6". The temperature was measured iron-rhodium resistance thermometer type RIRT 2 ($R_0 \approx 100$ Om). Absolute accuracy of temperature measurements ±5 10^{-3} K. The energy equivalent of the calorimeter was determined by measuring the heat capacity of the ampoule filled with helium to a pressure of 16 kPa. To check the reliability of the measured heat capacity of the calorimeter installation of standard samples of benzoic acid grade K-1 (Mendeleev All-Russian Scientific Research Institute of Metrology, St. Petersburg) and copper grade "ultrapure 11-4". Deviation of the values obtained from the heat capacity of the passport data and metrological work was 0.2 % in the region of 80-350 K.

Enthalpy of combustion BT ($\Delta_c H^o$) was determined in an improved calorimeter B-08MA with a static calorimeter bomb [11]. Calibration of calorimeter system was carried out by standard benzoic acid grade K-2 ($\Delta_c U = -26460.0$ J/g by weighing in the air). Energy equivalent of systems $W = 14805 \pm 3$ J/K with double standard deviation from the average value of 0.02%. Samples BT burned into tablets together with tablets of benzoic acid at an oxygen pressure of $3 \cdot 10^6$ Pa. By chromatographic analysis used oxygen contained impurities mol%: N_2 − 0.8; CO and CO_2 − 0.002; hydrocarbons − 0.001. Substance in a quartz crucible torched by capacitor discharge platinum wire connected to the product by cotton thread. To measure the temperature rise using two platinum resistance thermometer and a digital voltmeter included in a bridge circuit. Percentage ratio of the mass of CO_2 (experimental) to the mass of CO_2 (calculated) is for BT 99.97-100.03%. Calculations are performed for the following combustion reaction:

$$C_{30}H_{50}O_2\ (s) + 41.5\ O_2\ (g) \rightarrow 30\ CO_2\ (g) + 25\ H_2O\ (l) \qquad (1)$$

Under the conditions determined by bomb calorimeter energy combustion test substance Δ_cU. Based on the average value of $\Delta_c U$, molar mass (M), as amended by Washburn and amendments due to changes in the number of moles of gas, estimated a value of $\Delta_c U^o$ and $\Delta_c H^o$ reaction (1) combustion BT. According to the obtained $\Delta_c H^o$ calculated enthalpy of formation $\Delta_f H^o$ BT solid at 298.15 K using $\Delta_f H^o(H_2O, l) = -285.830 \pm 0.042$ J/mol and $\Delta_f H^o(CO_2, g) = -393.51 \pm 0.13$ J/mol [12].

Phase and relaxation transitions in samples of CO_2-extracts in the 80-650 K recorded DTA at the facility whose design and working procedure is described in [13]. Sample and standard sample were 0.1-0.3 g load them in hermetically glass or open aluminum crucibles. Served standard quartz. The experiment was conducted in a helium atmosphere. The heating rate in the tests was 5 K/min. Deviation from linearity does not exceed 1%. Temperature measurement accuracy chromel-copel thermocouple ±0.5 K. For the temperature relaxation transitions studied samples taken average temperature interval , as it is she has the best reproducibility [14].

RESULTS

Studies CO_2-extracts of pine shoot method of IR-spectroscopy showed that the spectrum of the hydrocarbon fraction contains absorption bands 2920, 2960, 2850, 1380, 1460 cm^{-1}, typical for C−H vibrations. IR-spectrum compounds carbonyl contains absorption bands 1730, 1250 cm^{-1}, characteristic for C=O group. Alcohols spectrum contains absorption bands 1730, 1250, 1040 cm^{-1} relating to oscillations of C=O and O−H groups of the alcohols and ether alcohols.

Gas-liquid chromatography method identified 9 of higher fatty and rosin acids 10 to carbon dioxide extracts of pine shoots. The maximum amount of vitamin F is 3.22%, and vitamin E − 10-50 mg/100 g was also determined the content of monoterpene hydrocarbons and neutral compounds in extracts of pine shoots. The extracts of pine shoot have large values of the acid number (74-119 mg KOH/g) and a small number of values ether (15-60 mg KOH/g).

We carried out a few samples DTA CO_2-extracts from pine shoots [8]. All samples were initially cooled with speed 20 K/min from room temperature to 80°C. Then, while heating the DTA-curves were recorded. Thermograms of all samples were shown the same relaxation and phase transitions. Upon initial warming in this sample as well as the others, manifested relaxation transition (T_{g1}) endothermic character in the range 240-255 K. Then, at 273 K exhibits an endothermic melting peak of the water phase and another relaxation transition (T_{g2}) in the range 300-320 K, after which the two endothermic peaks with extremes at T_{evap1} = 355 K and T_{evap2} = 380 K. The observed weight loss of the sample from 6 to 20 wt.%. This indicates that the last two peaks are related to the evaporation of water and some volatile organic compounds. Wherein the water insoluble components in the extract. Apparently, the extract and constituents do not dissolve in water, as its melting point is not lowered. After evaporation of volatile components, we again cooled samples and took second thermogram with heating. When again heating of the missing peaks in the thermograms of melting and evaporation of water. Relaxation transitions in the ranges 240-255 K and 300-320 K reproduced and appeared two transitions (T_{g3} and T_{g4}) with average temperatures of 353 and 378 K in the range of peaks evaporation. Registered relaxation transitions should be attributed to devitrification components included in the extracts. Their temperature can be used in the standardization of these samples, CO_2-extracts from pine shoots.

Our studies [14] suggests that the extract pine shoots has radioprotective properties, as evidenced by a higher content of bone marrow cells and white blood cells, as well as rapid recovery processes in the blood system and hematopoiesis in irradiated animals pre- treated with an extract of pine shoots, relative to control animals. Thus, it can serve as a promising radioprotective. Adding rats eating oily solution extract of pine shoot induced already within the first week a significant decrease in plasma total cholesterol, increasing high-density lipoprotein cholesterol, a significant reduction of atherogenic index. The detected changes of the parameters studied suggest the presence of carbon dioxide extracts from pine shoots anti-atherogenic properties.

To establish reproducibility relaxation and phase transitions in the sample CO_2-extract of birch bark experiment was repeated four times. Fig.2 shows the thermogram of the first three experiments. In experiments 1 and 2 (Fig.2, curves 1 and 2) heating finished at 400 K. Thermograms 1 and 2 appeared three consecutive relaxation transition (T_{g1}, T_{g2}, T_{g3}) endothermic character in the range 260-390 K, respectively, with average temperatures T_{g1} = 268, 269 K, T_{g2} = 319, 318 K, T_{g3} = 376, 373 K. In experiment 3 (Fig.2, curve 3) heating finished at 510 K. Thermogram 3 also appeared three relaxation transition in the range 275-390 K. However, the temperatures were higher than their 3-10 K as compared with the first two experiments. It should be noted that after the first two passes the sample has lost of ~ 2% of the original mass. It could be the adsorption water or other highly volatile low molecular weight components. As is well known [13, 15-17], low molecular weight compounds have a plasticizing effect on polymers, lowering the temperature of their relaxation transitions. Apparently, their evaporation of CO_2-extract of birch bark can be explained by an increase in temperature T_{g1}, T_{g2} and T_{g3} in the third experiment. In addition, the thermogram 3 fourth manifested relaxation transition (T_{g4}) in the range 410-430 K. Its amplitude was significantly larger than the first three. Then manifested large exothermic peak (T_{cr2} = 467 K), which can be attributed to the process of crystallization and endothermic peak (T_{m1} = 501 K), referring, apparently, to melt. Loss of weight of the sample after the third was warm ~0.5 wt.%. This indicates the absence of destructive processes in the sample.

However, it should be noted that heating to 510 K has a definite effect on the sample. This manifests itself in varying amounts and temperatures of relaxation transitions during the fourth experiment . Temperature $T_{g1} - T_{g4}$ dropped sharply, there was the fifth transition at T_{g5} = 450 K, another exothermic peak (T_{cr1} = 400 K) and the second endothermic process at T_{m2} = 514 K. The sample lost 7% of its weight in the previous experiment that indicates destructive processes in the sample during the last warming.

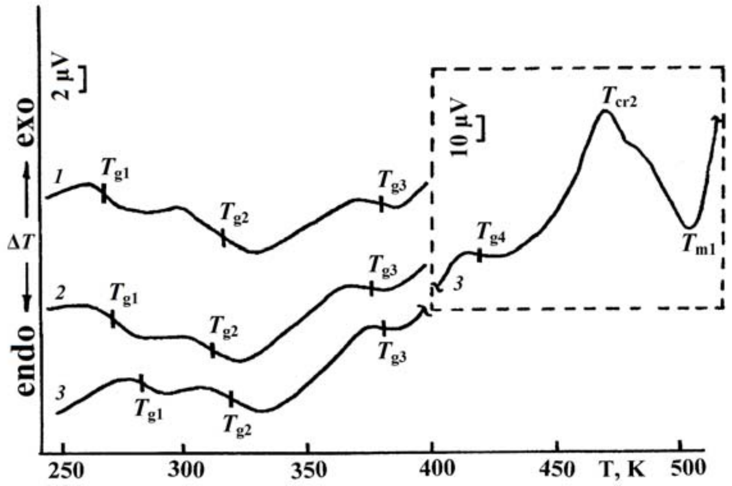

Fig.2. Thermogram of CO_2-extracts of birch bark (*Betula pendula*):
1 - the first heating; 2 - the second heating, 3 - the third heating

The experimental values of the heat capacity of BT presented in Fig.3. As can be seen from Fig.3, the heat capacity of BT increases monotonically in the range 80-230 K. Then the curve $C^\circ_p = f(T)$ relaxation transition manifested − an anomalous increase in the heat capacity. Such transitions in crystalline materials referred to glassy (G-type). The average temperature T_G = 235 K.

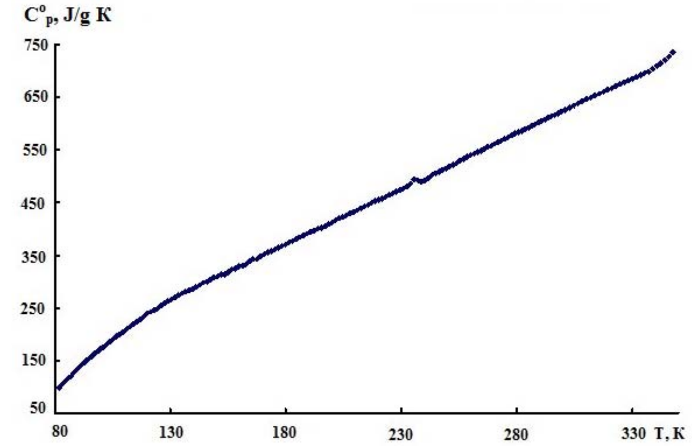

Fig.3. The experimental values of the molar heat capacity of betulin

DTA method to determine the temperature of phase transitions and relaxation BT (Fig.4). On its thermogram (Fig.4) in the range of 220-260 K, as well as on the heat capacity (Fig.3), manifested endothermic transition character − relatively sharp deviation from the zero baseline stroke associated with an increase in the heat capacity of the sample. Average temperature interval T_G = 240 K, which agrees well with the temperature determined calorimetrically. Then thermogram exotherm was observed (T_{cr} = 433 K), corresponding to a transition in the crystal (II), are stable in this temperature range. When the temperature T(CrII↔CrI) = 501 K crystals (II) transitions in crystals (I), melting at T_m = 530 K. These transitions are reversible, because reproduced in repeated sample heating. I.e. BT crystal modifications can be attributed to enantiotropic. The authors of [18] note the possible existence at BT several allotropic crystalline modifications.

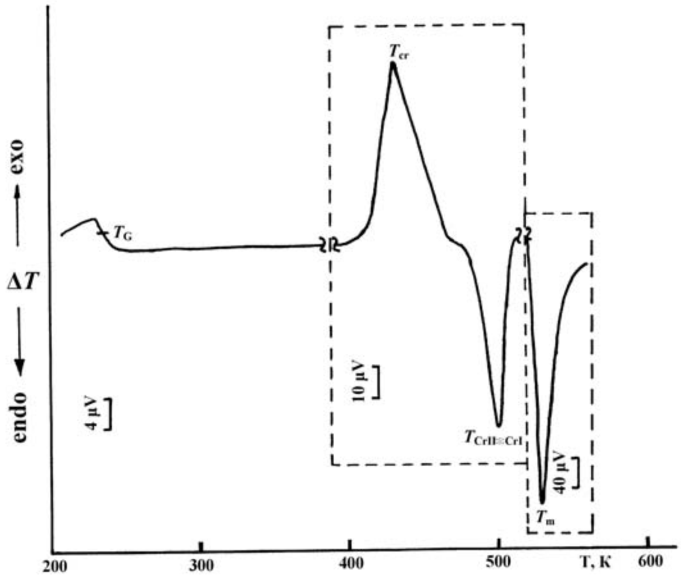

Fig.4. Thermogram of betulin

We have determined the energy of combustion of BT. At face value $\Delta_c U$ timed it $\Delta_c H^o$(BT, s.) = −17774.0±16.3 kJ/mol and $\Delta_f H^o$ (BT, s) = −1177.1±16.3 kJ/mol. Calculations are performed for the combustion reaction BT (1).

The results of thermal analysis of oils (No1 − No6) are presented in Table 1 and Fig.5.

Table 1. Temperature relaxation transitions of oils No1 – No6

Sample	No1	No2	No3	No4	No5	No6
Weight of sample, g	0.4055	0.0825	0.0045	0.5000	0.3251	0.1593
T_{g1}, K	215±2	230±1	252±1	245±1	197±1	255±1
T_{g2}, K	284±1	279±0.5	294±1	290±1	223±1	315±2
T_{cr}, K	----	----	----	----	247.5±0.5	-----
T_m, K	----	----	----	----	258.0±0.5	371±7

As can be seen from Table 1, in the thermograms of all the studied oils appeared two relaxation endothermic transition issues associated with an abnormal increase in the heat capacity of materials in a narrow temperature range. These include relaxation transitions to devitrification. Having two devitrification temperature ranges indicates a biphasic system. Delamination of the two phases and the formation occurs upon cooling oils. This was confirmed in the study of samples of visual polythermic method by Alekseev [19].

Comparing the two samples annual geranium oil (*Pelargonium graveolens*), obtained by different methods (CO_2-extraction − No2 and microwave extraction − No4) shows that they different in the glass transition temperature (Table 1). This indicates that the composition (qualitative or quantitative) of the phases different from them.

In pomegranate seed oil (Table 1, Fig.5) but two glass transition (T_{g1} = 197±1 K and T_{g2} = 223±1 K) manifested crystallization (T_{cr} = 247.5±0.5 K) and melting (T_m = 371±7 K). During these phase transitions is apparently responsible paraffins contained in sample No5.

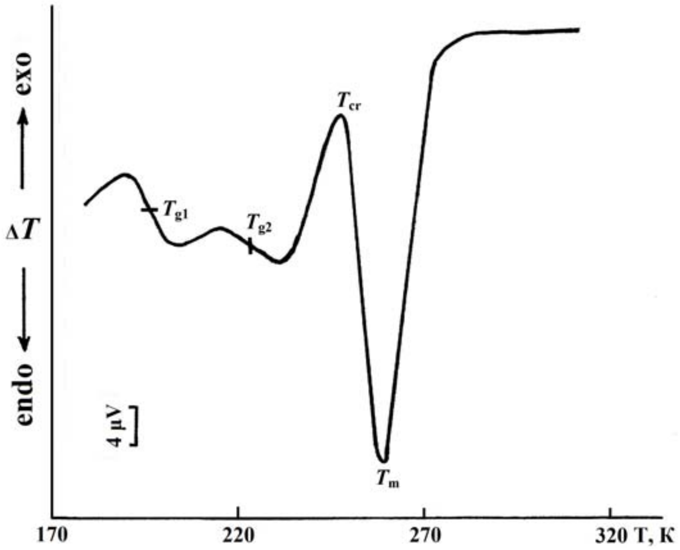

Fig.5. Thermogram of the sample (No5) pomegranate (*Punica granatum*) seed oil

At St. John's wort (*Hypericum perforatum*) oil (Table 1) was also demonstrated two temperature interval of devitrification (T_{g1} = 255±1 K and T_{g2} = 315±2 K) and melting (T_m = 258.0±0.5 K). Unlike pomegranate seed oil it immediately crystallized upon cooling.

CONCLUSION

The results indicate that the temperature of phase transitions and relaxation, registered DTA and calorimetry at BAS derived CO_2-extraction, can be successfully used for a qualitative analysis and standardization.

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